REMARKS

THE AMENDMENTS

Applicants have amended claims 27-32, 34, 43-44, 46 and 48, added claims 54-60 and canceled claims 35-38. Following entry of this amendment, claims 27-32, 34, 43-44, 46, 48, and 51-60 will be pending in this application.

Applicants have canceled claims 35-38 without prejudice or waiver of applicant's right to file for and obtain claims directed to any canceled subject matter in this application or in future divisional or continuing applications claiming priority from this application.

Applicants have amended claims 27-32, 34, 43-44, 46 and 48 to limit the morphogen to OP-1. Support for this amendment may be found, for example, at specification page 7, lines 6-7 and original claim 16.

Applicants have added claims 54-60. Support for new claims 54-60 may be found, for example, at specification page 6, lines 15-19; page 7, lines 6-7; page 8, lines 6-9; page 12, lines 11-12; page 17, lines 22-23; page 29, lines 5-10; page 40, lines 3-6 and original claim 16.

None of the amendments introduces any new matter.

THE REJECTIONS

35 U.S.C. § 112, First Paragraph - Enablement
Claims 27-32, 34-38, 43-44, 46, 48 and 51-53

The Examiner has rejected claims 27-32, 34-38, 43-44, 46, 48 and 51-53 under 35 U.S.C. § 112, first paragraph, for lack of enablement. The Examiner states that the specification, while being enabling for "up-regulating the expression of N-CAM and L1 in NG108-15 cells and increasing dendritic arbors of 7-14 DIV cultured hippocampal neurons with OP-1," does not provide enablement for a method for reducing spatial or declarative memory dysfunction caused by damaged hippocampal tissues and caused by permanent or global ischemia comprising determining the existence of spatial or declarative memory dysfunction and administering a structurally ill-defined morphogen merely comprising a conserved C-terminal seven-cysteine skeleton that is at least about 60% identical or 70% homologous to residues 330-431 of human OP-1 or fragments thereof. The Examiner contends that memory dysfunction or deficit is a complex process and its cause or process is not clear. The Examiner states that it is unpredictable whether the claimed method can truly reduce memory dysfunction. The Examiner further contends that neither the specification nor the prior art show that the claimed morphogens with limited homology are effective in treatment of reduced memory dysfunction, and that it is unpredictable whether these claimed morphogens would truly work in the claimed method.

Applicants traverse. However, solely to expedite prosecution of this application, applicants have canceled claims 36-38 and amended claims 27, 46 and 48 (and therefore, claims dependent therefrom) to limit the morphogen to OP-1. Applicants respectfully submit that the claims, as amended, are fully enabled

by applicants' specification.

Applicants have demonstrated that OP-1 induces N-CAM expression in NG108-15 cells (see page 47, line 5 to page 59, line 6). In addition, applicants have demonstrated that the addition of OP-1 to cultured hippocampal neurons significantly accelerates dendritic outgrowth and development and increase the number of synapses as compared to untreated cells (see page 59, line 9 to page 61, line 7). And, applicants have demonstrated that treatment of hippocampal neurons with OP-1 resulted in enhanced expression of MAP2, a marker of dendrite morphogenesis, demonstrating the ability of OP-1 to stimulate dendritic morphogenesis (see page 61, lines 1-7). The specification also describes that "cell loss in the hippocampus affects both 'spatial memory' or 'spatial learning',... and general memory function, also referred to as 'declarative memory'" (*see*, page 48, lines 10-18). Dendrite density has been demonstrated to correlate with retention of memory function in normal aging brain (Morrison et al., of record).

Applicants also submit herewith Li et al., J Neurosci Res., 87(1):112-22 (2009) ("Li", Appendix A) as post filing evidence that hippocampal neurogenesis attenuates spatial cognitive deficits induced by ischemic stroke. Specifically, Li demonstrated that fluoxetine treatment (10 mg kg(-1), i.p.) for 4 weeks promoted the survival of newborn cells in the ischemic hippocampus and, consequently, attenuated spatial memory impairment of mice after focal cerebral ischemia (see Figure 4). Disrupting hippocampal neurogenesis blocked the beneficial effect of fluoxetine on ischemia-induced spatial cognitive impairment (see Figure 8). Thus, Li confirms that hippocampal neurogenesis is involved in improving spatial cognitive deficits.

As described above, applicants have demonstrated that OP-1 enhances hippocampal dendritic outgrowth and morphogenesis. Therefore, based on the teachings of the instant application a person of ordinary skill in the art would be able to practice the claimed methods of reducing memory dysfunction *in vivo* by treating with OP-1.

For all of the above reasons, applicants respectfully request that the Examiner withdraw this rejection.

35 U.S.C. § 112, First Paragraph - Written description

Claims 34-35

The Examiner has rejected claims 34-35 under 35 U.S.C. § 112, first paragraph, for lack of written description. Specifically, the Examiner contends that the recitation of mature OP-1 comprising residues 293-431 of SEQ ID NO:2 constitutes new matter. Applicants traverse.

First applicants have canceled claim 35, thus obviating the rejection with respect to that claim. Second, the specification provides adequate written description for mature OP-1 comprising residues 293-431 of SEQ ID NO:2. At page 1, lines 24-26, the specification discloses that the members of the TFGβ superfamily are processed from a precursor "pro-form" to yield a mature polypeptide chain competent to dimerize and containing a carboxy terminal active domain, typically in the range of 97-106 amino acids. The specification at page 16, lines 3-14 also discloses that in their mature form, morphogenic proteins including OP-1, are dimers with a molecular weight of about 30-36 kD and that the proteins are translated as a precursor, having an N-terminal signal peptide sequence typically less than about 30 residues,

followed by a "pro" domain that is cleaved to yield the mature C-terminal domain. At page 29, lines 8-9, the specification defines the full "pro" sequence to be residues 30-292 of SEQ ID NO:2. Therefore, cleavage of the signal sequence, which is about 30 residues and cleavage of the pro region, which is residues 30-292 of SEQ ID NO:2, yields a polypeptide having residues 293-431 of SEQ ID NO:2.

Further, applicants note that several documents are identified in the instant application and which are incorporated by reference also identify mature OP-1 as residues 293-431 of SEQ ID NO:2. For example, US Patent No. 5,650,276 ("the '276 patent") (incorporated by reference in the instant application at page 37, lines 17-21) discloses that mature OP-1 is defined by SEQ ID NO:5 of that application. A comparison of the sequence set forth in SEQ ID NO:5 of the '276 patent with residues 293-431 of SEQ ID NO:2 of the instant application reveals that they are the same sequence.

For all the above reasons, applicants request that the Examiner withdraw the written description rejection.

Obviousness-type Double Patenting
Claims 27-32, 34-38, 43 and 44

The Examiner has rejected claims 27-32, 34-38, 43 and 44 under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-30 of U.S. Patent No. 6,407,060 ("the '060 patent"). The Examiner states that claims 1-30 of the '060 patent encompass a method for enhancing recovery of CNS function by a morphogen comprising a conserved C-terminal seven cysteine skeleton having at least 70% homology to amino acids 330-431 of hOP-1 in a mammal suffering from a CNS

injury caused by ischemia or trauma. The Examiner states that the neuronal damage in the instant claims is caused by ischemia, which is identical to the claims of the '060 patent. Further, the Examiner states that the claims of the instant application and the '060 patent encompass the same material and the same patient population which means that patients suffering from ischemia would also suffer from memory dysfunction.

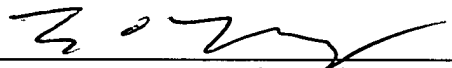
Applicants request that the obviousness-type double patenting be held in abeyance until allowable subject matter is found in the instant application. Applicants stand ready to address the obviousness-type double patenting rejection in an appropriate manner, i.e., by argument or by filing an appropriate terminal disclaimer, upon an indication by the Examiner that the claims of the instant application are otherwise allowable.

CONCLUSION

In view of the foregoing amendments and remarks, applicants request that the Examiner reconsider and withdraw all outstanding rejections and allow the pending claims.

The Examiner is invited to telephone applicants' representatives regarding any matter that may be handled by telephone to expedite allowance of the pending claims.

Respectfully submitted,



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Appendix A

Journal of Neuroscience Research 87:112–122 (2009)

Chronic Fluoxetine Treatment Improves Ischemia-Induced Spatial Cognitive Deficits Through Increasing Hippocampal Neurogenesis After Stroke

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Cognitive deficits, including spatial memory impairment, are very common after ischemic stroke. Neurogenesis in the dentate gyrus (DG) contributes to forming spatial memory in the ischemic brain. Fluoxetine, a selective serotonin reuptake inhibitor, can enhance neurogenesis in the hippocampus in physiological situations and some neurological diseases. However, whether it has effects on ischemia-induced spatial cognitive impairment and hippocampal neurogenesis has not been determined. Here we report that fluoxetine treatment (10 mg kg^{-1}, i.p.) for 4 weeks promoted the survival of newborn cells in the ischemic hippocampus and, consequently, attenuated spatial memory impairment of mice after focal cerebral ischemia. Disrupting hippocampal neurogenesis blocked the beneficial effect of fluoxetine on ischemia-induced spatial cognitive impairment. These results suggest that chronic fluoxetine treatment benefits spatial cognitive function recovery following ischemic insult, and the improved cognitive function is associated with enhanced newborn cell survival in the hippocampus. Our results raise the possibility that fluoxetine can be used as a drug to treat post-stroke spatial cognitive deficits.

Key words: focal cerebral ischemia; fluoxetine; hippocampus; neurogenesis; sensorimotor function; spatial cognitive function

Spatial cognitive deficits including spatial memory impairment are very common after ischemic stroke (Kidwell et al., 2001). Spatial memory is largely dependent on hippocampal formation (Morris et al., 1982; Squire, 1992; Tsien et al., 1996). Neurogenesis in the adult dentate gyrus (DG) is important in learning and memory processes (Kempermann et al., 1997; van Praag et al., 2002; Drapeau et al., 2003). In adult rodents, experimental cerebral ischemia enhances neurogenesis in the brain's neuroproliferative zones, the subgranular zone (SGZ) of the hippocampal DG (Liu et al., 1998; Tureyen et al., 2004; Tonchev and Yamashima, 2006) and the subventricular zone (SVZ) of the lateral ventricle (Jin et al., 2001; Tonchev et al., 2005). There is also evidence for stroke-induced neurogenesis in the human brain (Jin et al., 2006). Recently, these newborn neurons after ischemic injury were shown to migrate to injured brain regions (Schmidt and Reymann, 2002; Zhang et al., 2007), become actively integrated into the existing circuitry, and form appropriate synapses, which contributed to ameliorating neurological deficits and forming hippocampal-dependent memory (Nakatomi et al., 2002; Bendel et al., 2005). These studies raise the possibility that spatial cognitive deficits after ischemic stroke may be improved through enhancing hippocampal neurogenesis. However, the therapeutic potential of neurogenesis stimulated by cerebral ischemia itself is very limited, because many of the newly generated cells undergo physiological cell death, and the survival rate of newborn cells is low (Takasawa et al., 2002; Sun et al., 2003). Therefore, a precise understanding of the mechanism of neuronal regeneration and stimulation of hippocampal neurogenesis in the adult brain should contribute to devising novel strategies to treat stroke patients with spatial cognitive deficits.

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Neurogenesis in the adult brain is a plastic event influenced, at least in part, by several factors, including genetic background, gender, aging, hormones, growth factors, stress, environment, and exercise (Gage et al., 1998). Fluoxetine is a widely used antidepressant compound, whose primary action is based on the inhibition of serotonin reuptake in the central nervous system (CNS; Cipriani et al., 2005). Recent studies have shown that chronic fluoxetine treatment can promote the proliferation as well as the survival and differentiation of newborn cells in the hippocampus of the adult mammalian brain (Malberg et al., 2000) and in some neurological diseases, such as depression, in which hippocampal neurogenesis was proved to be requisite for the behavioral effects of fluoxetine (Santarelli et al., 2003; Sairanen et al., 2005). Furthermore, chronic fluoxetine treatment stimulates neurite outgrowth, dendritic branching, and neuroplasticity in the brain. The latter effect of fluoxetine has been referred to as *neuronal remodeling* (Horsfield et al., 2002). It is well established that CREB phosphorylation and the brain-derived neurotrophic factor (BDNF) expression pathway are highly important molecular mechanisms for the effects of chronic fluoxetine treatment (Russo-Neustadt et al., 2000; Tiraboschi et al., 2004). It is important to determine whether chronic fluoxetine treatment can enhance neurogenesis and neuronal remodeling in the hippocampus and improve spatial cognitive functional recovery after ischemic stroke, because this question remains to be answered. Aiming to determine the contribution of fluoxetine on ischemic stroke, we examined the effects of a chronic fluoxetine regimen on hippocampal neurogenesis and functional recovery, especially spatial cognitive function after focal cerebral ischemia.

MATERIALS AND METHODS

Animals and Transient Focal Cerebral Ischemia

The animal experiment protocol conformed to the NIH *Guide for the care and use of laboratory animals* and was approved by the Institutional Animal Care and Use Committee of Nanjing Medical University. Adult male C57/BL/6 mice, weighing 25–30 g, were used in this study.

Transient focal cerebral ischemia was induced by intraluminal middle cerebral artery occlusion (MCAO), as described previously (Zhu et al., 2003). In brief, with animals under chloral hydrate anesthesia (350 mg kg^{-1}, i.p.), an 8/0 surgical nylon monofilament with rounded tip was introduced into the left internal carotid artery through the external carotid stump and advanced 16–17 mm past the carotid bifurcation until a slight resistance was felt. At this point, the intraluminal filament blocked the origin of the MCA and occluded all sources of blood flow from the internal carotid artery, anterior cerebral artery, and posterior cerebral artery. Throughout the procedure, body temperature was maintained at 37°C ± 0.5°C. The local cerebral blood flow was monitored in the front parietal cortex of the occluded side by using Perimed PF5050 (Sweden) multichannel laser doppler flowmetry. The filament was left in place for 60 min and then withdrawn. In the sham-operated animals, the occluding filament was inserted only 7 mm above the carotid bifurcation.

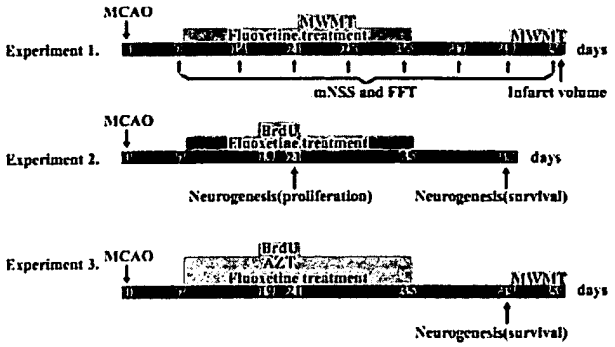

Fig. 1. Experimental design. Time points represent days after MCAO induction. Fluoxetine was administered during days 8–35. In experiment 1, mNSS and foot fault test were performed at every time point. The water maze test was performed during days 22–28 and days 50–59 after MCAO. In experiment 2, BrdU (50 mg kg^{-1}, i.p.) was given twice daily during days 19–21. BrdU immunohistochemistry was performed on day 21 and day 49 after MCAO. In experiment 3, AZT was administered during the period of fluoxetine treatment (day 8–35), and BrdU (50 mg kg^{-1}, i.p.) was given twice daily during days 19–21. BrdU immunohistochemistry was performed on day 49 after MCAO; water maze test was performed during day 50–59 after MCAO. MCAO, middle cerebral artery occlusion; MWMT, Morris water maze test; mNSS, modified neurological severity score; FFT, foot fault test; BrdU, 5-bromo-2′-deoxyuridine.

Experimental Protocol and Drug Treatment

The design of the experiments is illustrated in Figure 1. One week prior to surgery, mice were housed in standard laboratory cages (30 × 18 × 20 cm, four or five mice per cage) at a temperature of 20–21°C, with lights on 06:00–18:00 hr and with free access to food and water. On day 0, mice were subjected to MCAO and then returned to standard cages after 24 hr of recovery in individual cages. Behavioral assessments (see below) were made on day 7 after surgery to give the animal sufficient time to recover from surgery. The mice with similar behavioral impairment were randomly divided into three consecutive experiments. 1) To determine whether fluoxetine treatment improves sensorimotor functional recovery and attenuates spatial memory impairment, mice received i.p. injections of fluoxetine (dissolved in 0.9% NaCl and administered at a dose of 10 mg kg^{-1}) daily starting on day 8 after ischemia induction and continuing to 35 days after MCAO (28 days). The dose was selected based on previous reports (Windle and Corbett, 2005; Sairanen et al., 2005). Vehicle control mice and sham-operated mice were given an equivalent volume of vehicle (saline). Neurological severity scores and foot fault tests were performed 7, 14, 21, 28, 35, 42, 49, and 59 days after MCAO, and spatial cognitive performance was tested in the Morris water maze during days 22–28 and days 50–59 after MCAO. The design of this experiment included a 14-day withdrawal following 28-day of chronic fluoxetine treatment (from day 36 to day 49 after MCAO) before we performed the Morris water maze test for the last time. The purpose of selecting this paradigm is to exclude the possibility of acute responses of fluoxetine treatment on behavioral or cognitive performance and to reflect

the effects of hippocampal neurogenesis on cognitive performance. 2) To examine whether fluoxetine increases neurogenesis, mice received i.p. injections of 5-bromo-2′-deoxyuridine (BrdU; Sigma, St. Louis, MO) 50 mg kg^{-1} twice daily during days 19–21 after MCAO. This period was chosen because several studies indicated that at least 11 days were needed of fluoxetine treatment that could significantly increase BrdU-positive cells, and the extent of the increase did not differ from 28 days of treatment (Santarelli et al., 2003). For the cellular proliferation study, animals were sacrificed 12 hr after the last BrdU injection to examine the number of newly formed cells in the DG. To determine the survival and cell phenotypes of the newly born cells, animals were sacrificed 28 days after the last BrdU injection. 3) Telomerase, a reverse transcriptase that maintains chromosome ends (telomeres) during successive cell divisions in mitotic cells, is present in neuroblasts and early postmitotic embryonic neurons but is absent from most somatic cells in the adult (Klapper et al., 2001). It plays an important role in maintaining the cells in a proliferative state and is required for the long-term survival of early postmitotic neurons (Fu et al., 2002). To determine whether hippocampal neurogenesis is necessary for the effect of fluoxetine on spatial cognitive performance after MCAO, we used a telomerase inhibitor, 3′-azido-deoxythymidine (AZT), to disrupt neurogenesis. The mice were treated with 100 mg/kg AZT per day i.p. during the period of fluoxetine treatment (from day 8 to day 35 after MCAO). The dose of AZT used was based on our previous report (Zhou et al., 2007). Spatial cognitive performance and hippocampal neurogenesis were tested 49 days after MCAO (28 days after the last BrdU administration) by the same approach as mentioned previously.

Neurological Severity Scores

Modified neurological severity score (mNSS) is a composite of motor, sensory, reflex, and balance tests (Chen et al., 2001). This score is derived by evaluating animals for hemiparesis (response to raising the mice by the tail or placing the mice on a flat surface), abnormal movements (immobility, tremor, seizures), sensory deficits (placing, proprioception), and absent reflexes (pinna, corneal, startle). Neurological function was graded on a scale of 0 to 18 (normal score 0; mild injury 1–6; moderate injury 7–12; severe injury 13–18).

Foot Fault Test

Mice were tested for forelimb placement dysfunction with the foot fault test (Gibson et al., 2005). Mice were placed on an elevated grid surface whose grid openings were 2.5 cm^2. During locomotion on the grid, the number of foot faults made by the left and right forelimbs was counted. Each test consisted of three trials lasting 1 min each, with an interval of 1 min. Foot faults are expressed as the number of errors made by the contralateral (left) forelimb as a percentage of the total errors made.

Morris Water Maze Task

The spatial cognitive performance of mice was evaluated by the Morris water maze test (Morris, 1984). A circular swimming pool (Neuroscience Inc.) measuring 138 cm in diameter and 45 cm in height was filled with opaque water made by white nontoxic paint to a depth of 33 cm at 24°C ± 2°C. Four starting points around the edge of the pool were designated as N, E, S, and W, which divided the pool into four quadrants. A platform, 10 cm in diameter, was located in a constant position in the middle of one quadrant. To render it invisible to the mice, the platform was submerged 1.2 cm below the surface of the water. The task for the mice was to escape from the water by locating the hidden platform. Two days before training, the animals were habituated to swimming for 60 sec in the pool without a platform. One block of four trials was given for 6 consecutive days. For each trial, the mouse was placed in the water facing the wall of the pool at one of four starting points and allowed to swim for a maximum of 90 sec. If the mice found the platform, they were allowed to remain on it for 10 sec; the mice not finding the platform were guided to it and allowed to remain there for 10 sec. Each trial was videotaped via a ceiling-mounted video camera, and the animal's movement was tracked using Ethovision software, which allows the calculation of various measures such as latency and swimming length and speed. On the seventh day, mice were given one 90-sec retention probe test in which the platform was removed from the pool. During retention, the number of times each animal crossed the area in which the platform had previously been located and the time spent in the target quadrant were measured. After probe test, a visible platform trial was started to examine possible nonspatial memory. During the visible platform trials, the platform was elevated 0.5 cm above the water level and marked by a yellow label. The location of the visible platform varied for each trial. Four trials were administered each day, and the test lasted for 3 days. The latency to reach the visible platform was measured. All Morris water maze tests were performed between 08:00 and 12:00 AM.

Immunohistochemistry and Cell Counting

The animals were anesthetized with chloral hydrate (350 mg kg^{-1}, i.p.) and perfused transcardially with saline, followed by 4% paraformaldehyde. Brains were removed and postfixed overnight in the same solution. Serial sections (40 μm) were made on an oscillating tissue slicer in a bath of physiological saline. Every fifth section throughout the hippocampus or SVZ was processed for BrdU immunohistochemistry as described previously (Zhu et al., 2003). The sections were heated (85°C for 5 min) in an antigen unmasking solution (Vector Laboratories, Burlingame, CA); incubated in 2 M HCl (30°C for 30 min), rinsed in 0.1 M boric acid, pH 8.5, for 10 min; incubated in 1% H_2O_2 in phosphate-buffered saline (PBS) for 30 min; and blocked in PBS containing 3% normal goat serum, 0.3% (w/v) Triton X-100, and 0.1% bovine serum albumin (BSA; room temperature for 1 hr), followed by incubation with rat monoclonal anti-BrdU (1:200; Accurate Chemical and Scientific Corporation, Westbury, NY) at 4°C overnight. Subsequently, the sections were developed with the ABC Kit (Vector Laboratories). For double labeling, the sections were incubated with primary antibodies rat anti-BrdU (1:200) and mouse anti-NeuN (1:100; Chemi-

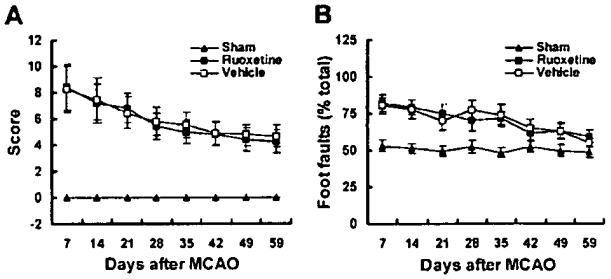

Fig. 2. Effects of fluoxetine treatment on sensorimotor functional recovery after MCAO. Behavioral tests (mNSS, foot fault test) for sensorimotor function were performed 7, 14, 21, 28, 35, 42, 49, and 59 days after MCAO. **A:** mNSS in sham-operated (n = 12), vehicle-treated (n = 12), and fluoxetine-treated (n = 12) animals after MCAO. There was no significant difference between vehicle- and fluoxetine-treated groups throughout the entire period (repeated-measures two-way ANOVA). **B:** Foot fault test of sham-operated (n = 12), vehicle-treated (n = 12), and fluoxetine-treated (n = 12) animals after MCAO. Unilateral foot faults were expressed by the number of contralateral foot faults as a percentage of the total errors made. The fluoxetine treatment group did not significantly differ from the ischemic control group in this functional deficit. Data are expressed as mean ± SEM.

con) or mouse anti-GFAP (1:1,000; Sigma) at 4°C overnight, then incubated with secondary antibodies goat anti-rat Cy3 (1:200; Chemicon) and goat anti-mouse FITC (1:50; Chemicon) for 2 hr at room temperature.

An experimenter coded all slides from the experiments before quantitative analysis. All BrdU-labeled cells in the DG or SVZ were counted in each section by another experimenter blinded to the study code. The total number of BrdU-labeled cells per section was determined and multiplied by 5 to obtain the total number of cells per DG. Number of double-stained $BrdU^+/NeuN^+$ and $BrdU^+/GFAP^+$ cells was analyzed by sampling every section from the experimental animals with a fluorescence microscope as described previously (Zhu et al., 2003).

Statistical Analyses

Results are expressed as means ± SEM. Repeated-measures two-way ANOVA was used to evaluate the results of experiments conducted over a series of days (i.e., mNSS, foot fault test, and Morris water maze test), followed by LSD test for post hoc analysis. Cognitive data from the probe trials in Morris water maze test and other data were analyzed by one-way ANOVA. LSD test for post hoc analysis was used when appropriate. The criterion for statistical significance was $P < 0.05$.

RESULTS

Fluoxetine Does Not Improve Sensorimotor Functions

To evaluate the effects of chronic administration of fluoxetine on sensorimotor functional recovery, mice were treated with fluoxetine once per day for 28 days, and their sensorimotor functions were measured once per week for 8 weeks beginning from day 8 after MCAO. A set of behavioral tests was performed in mNSS, including motor, sensory, reflex, and balance tests. Although the animals subjected to MCAO exhibited significant and sustained neurological deficits compared with sham-operated mice, the mean modified neurological severity scores showed no significant difference between fluoxetine- and vehicle-treated mice ($F_{1,22} = 0.182$, $P > 0.05$) throughout the testing period (Fig. 2A). Unilateral foot faults were expressed by the number of contralateral foot faults as a percentage of the total errors made (Fig. 2B), and a value of 50% represents an equal number of errors made by both sides. In sham-operated animals, no functional deficit was observed; they made approximately the same number of errors on the contralateral side as they did on the ipsilateral side. In the mice subjected to MCAO, there was a significant increase in the number of contralateral errors ($F_{2,33} = 113.440$, $P < 0.01$). However, fluoxetine treatment did not significantly reduce this functional deficit compared with vehicle ($P > 0.05$). These results suggest that chronic fluoxetine treatment does not significantly improve sensorimotor functional recovery after stroke.

Fluoxetine Attenuates Spatial Cognitive Deficits

The spatial cognitive ability was tested in the Morris water maze. To examine the effect of chronic fluoxetine treatment on spatial learning and memory, we first exposed animals to water maze task after fluoxetine treatment for 14–20 days (day 22–28 after MCAO). In the hidden platform trials, with regard to escape latency (Fig. 3A), repeated-measures two-way ANOVA revealed a group difference ($F_{2,33} = 8.379$, $P < 0.01$). Post hoc analysis using LSD test revealed that sham-operated group had a significantly reduced escape latency compared with the two ischemic groups, with vehicle ($P < 0.01$) and with fluoxetine ($P < 0.01$). However, there was no significant difference between fluoxetine- and vehicle-treated mice in escape latency ($P > 0.05$). Ischemic groups had significantly prolonged swimming length also compared with sham-operated mice ($F_{2,33} = 7.540$, $P < 0.01$; Fig. 3B). Repeated-measures two-way ANOVA of the swimming speed showed that there were no differences among the three groups ($F_{2,33} = 3.043$, $P > 0.05$; Fig. 3C), suggesting that the impaired spatial cognitive ability of ischemic mice on the water maze task is not caused by motor ability changes. During the spatial probe trials, in which the platform was removed, ischemic mice exhibited reduced time spent in the target quadrant ($P < 0.01$; Fig. 3D) and number of crossing platform position ($P < 0.05$; Fig. 3E) compared with sham-operated mice (one-way ANOVA). Therefore, fluoxetine treatment for 14 days does not ameliorate the MCAO-induced spatial learning and memory impairment.

When animals were exposed to the water maze task after 28 days of fluoxetine treatment and 14 days of drug withdrawal, however, the escape latency in fluoxetine-treated group was significantly shorter than that in

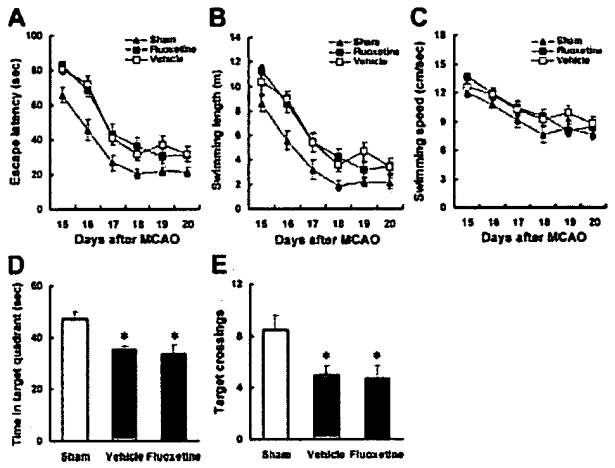

Fig. 3. Effects of 14 days fluoxetine treatment on spatial cognitive deficits after MCAO. Spatial cognitive performance of sham-operated (n = 12), vehicle-treated (n = 12), and fluoxetine-treated (n = 12) animals was tested in the Morris water maze after 14 days fluoxetine treatment (days 22–28 after MCAO). Fluoxetine treatment for 14 days did not ameliorate the marked deficits in spatial cognitive function induced by MCAO; there were no significant difference between vehicle- and fluoxetine-treated groups in escape latency (**A**) or swimming length (**B**) in the hidden platform trails, time spent in the target quadrant (**D**), or number of crossing platform position (**E**) in the spatial probe trails. There were no differences in the swimming speed (**C**) among the three groups in the hidden platform trails. Repeated-measures two-way ANOVA for the data from hidden platform trails and one-way ANOVA for the data from spatial probe trails, followed by post hoc LSD test. Data are expressed as mean ± SEM. $^*P < 0.05$ compared with sham-operated group.

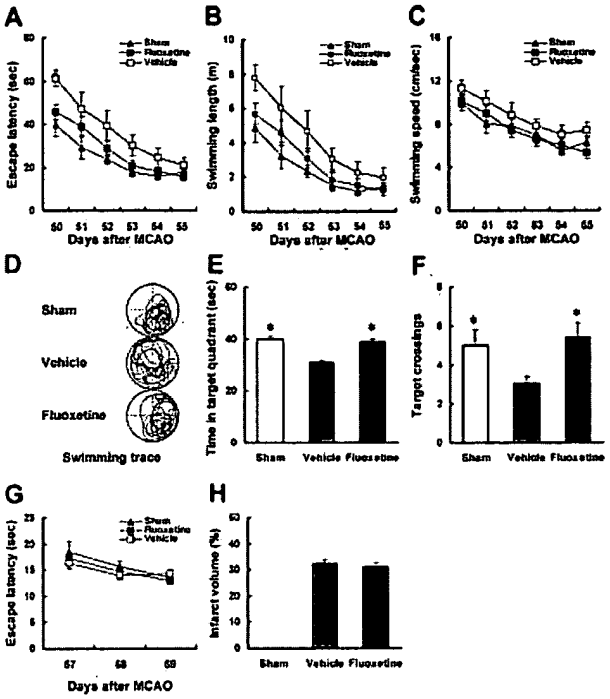

Fig. 4. Effects of 28 days of fluoxetine treatment on spatial cognitive deficits after MCAO. Spatial cognitive performance of sham-operated (n = 12), vehicle-treated (n = 12), and fluoxetine-treated (n = 12) animals was tested in the Morris water maze after 28 days of fluoxetine treatment and 14 days of drug withdrawal (days 50–59 after MCAO). Fluoxetine treatment for 28 days attenuated the marked deficits in spatial cognitive function induced by MCAO; the fluoxetine-treated group had reduced escape latency (**A**) and swimming length (**B**) in the hidden platform trails and increased time spent in the target quadrant (**E**) and number of crossing platform position (**F**) in the spatial probe trails than in vehicle-treated group, which was similar to that of sham-operated group. There were no differences in the swimming speed (**C**) among the three groups in the hidden platform trails. **D:** Typical swimming trace in the spatial probe trails for each group. **G:** The escape latency in the visible platform trails showed no significant differences among the three groups. **H:** Infarct volume demonstrated no significant difference between vehicle- and fluoxetine-treated groups. Repeated-measures two-way ANOVA for the data from hidden platform trails and visible platform trails and one-way ANOVA for the data from spatial probe trails and infarct volume followed by post hoc LSD test. Data are expressed as mean ± SEM. $^*P < 0.05$ compared with vehicle-treated group.

the vehicle-treated group ($P < 0.05$) and was similar to that in the sham-operated group ($P > 0.05$; Fig. 4A). Similarly, fluoxetine-treated mice had significantly reduced swimming length compared with vehicle-treated mice (Fig. 4B). Swimming speed was also measured (Fig. 4C), and there were no significant differences between groups ($F_{2,33} = 2.301$, $P > 0.05$). In the spatial probe trials, the swimming traces of sham-operated and fluoxetine-treated mice concentrated in the target zone where the platform had been set. However, the swimming traces of vehicle-treated mice uniformly distributed around four zones (Fig. 4D). Sham-operated and fluoxetine-treated mice exhibited markedly increased time spent in the target quadrant (Fig. 4E) and number of crossing the platform position (Fig. 4F) compared with vehicle-treated mice ($P < 0.05$). To exclude the possibility that the improved memory by chronic fluoxetine treatment was confounded by motivational or sensorimotor factors, we performed a water maze task with visible platform in which the platform was elevated 0.5 cm above the water level. Repeated-measures two-way ANOVA of escape latency (Fig. 4G) demonstrated no significant differences between groups ($F_{2,33} = 0.774$, $P > 0.05$), suggesting that the improved memory by fluoxetine is spatial memory. At the end of behavioral tests, the infarct volume was determined in mice from the three groups by TTC staining (Zhu et al., 2003). Fluoxetine treatment for 4 weeks starting day 8 after MCAO did not lessen infarct volume ($P > 0.05$), suggesting that the improved memory with fluoxetine is not due to infarct volume reduction (Fig. 4H).

Fluoxetine Promotes the Survival of Newborn Cells in the Dentate Gyrus

Neurogenesis in the adult DG contributes to hippocampus-dependent memory (Shors et al., 2001). To

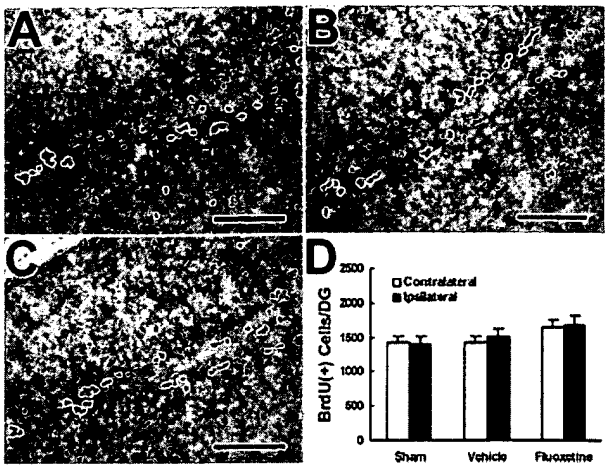

Fig. 5. Effects of fluoxetine treatment on newborn cells proliferation in the DG after MCAO. The mice received BrdU injection twice daily during days 19–21 after MCAO (days 12–14 after fluoxetine treatment). Cell proliferation was estimated by $BrdU^+$ cells 12 hr after the last BrdU injection. Representatives of the $BrdU^+$ cells in the DG of the sham-operated (**A**), vehicle-treated (**B**), and fluoxetine-treated (**C**) mice. **D:** Total number of $BrdU^+$ cells in the ipsilateral and contralateral DG of sham-operated (n = 5), vehicle-treated (n = 4), and fluoxetine-treated (n = 5) groups. Fluoxetine treatment for 14 days did not significantly increase progenitor cells proliferation in the DG of ischemic brain; there was no significant difference in the numbers of $BrdU^+$ cells among the three groups in either hemisphere (one-way ANOVA). Data are expressed as mean ± SEM. Scale bars = 100 μm.

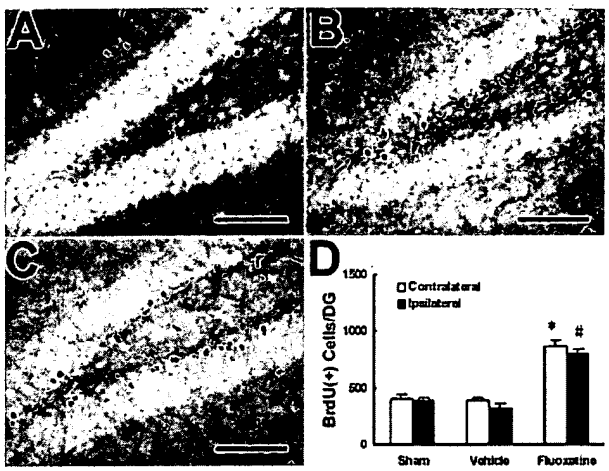

Fig. 6. Effects of fluoxetine treatment on newborn cell survival in the DG after MCAO. The survival of newborn cells was examined 28 days after the last BrdU injection. Representatives of the $BrdU^+$ cells in the DG of the sham-operated (**A**), vehicle-treated (**B**), and fluoxetine-treated (**C**) mice. **D:** Total number of BrdU-labeled cells in the ipsilateral and contralateral DG of sham-operated (n = 5), vehicle-treated (n = 4), and fluoxetine-treated (n = 4) groups. Fluoxetine treatment significantly increased newborn cells survival in the DG of ischemic brain, as the number of $BrdU^+$ cells in the DG of either cerebral hemisphere was significantly increased in the animals treated with fluoxetine compared with vehicle- treated or sham-operated animals (one-way ANOVA). Data are expressed as mean ± SEM. $^*P < 0.01$ compared with contralateral DG of vehicle-treated or sham-operated group; $^{\#}P < 0.01$ compared with ipsilateral DG of vehicle-treated or sham-operated group. Scale bars = 100 μm.

determine whether the spatial memory improvement with chronic fluoxetine treatment is dependent on enhanced neurogenesis in the DG, we first examined progenitor cell proliferation in the mice treated with fluoxetine or vehicle for 14 days (day 8 to day 21 after MCAO) by BrdU labeling. The number of BrdU-positive cells in the DG of fluoxetine-treated mice did not differ from that of vehicle both ipsilaterally and contralalterally ($P > 0.05$), suggesting that chronic fluoxetine treatment has no effect on progenitor cell proliferation in the DG of ischemic mice (Fig. 5B–D). Neither fluoxetine nor vehicle treatment changed the number of BrdU-positive cells in the DG of ischemic mice compared with sham-operated mice in either the ipsilateral or the contralalteral region (Fig. 5A,D). Moreover, there was no significant difference in the numbers of BrdU-positive cells between the ipsilateral and the contralateral sides in fluoxetine-, vehicle-treated, or sham-operated groups (Fig. 5D).

Newly born cells in the hippocampus can have several fates. Some cells die, whereas others survive and differentiate into mature neurons or glial cells. To examine the influence of fluoxetine treatment on cell fate, we treated mice with fluoxetine for 28 days (days 8–35 after MCAO) and BrdU for 3 days (days 19–21 after MCAO). The mice were killed 28 days after the last BrdU administration. The number of BrdU-positive cells both in the ipsilateral and in the contralateral DG was significantly increased in the animals treated with fluoxetine compared with vehicle ($P < 0.01$) or sham-operated mice ($P < 0.01$), suggesting that chronic fluoxetine treatment increased the survival of newly born cells in the hippocampus after stroke (Fig. 6). There was no significant difference in the numbers of BrdU-positive cells between vehicle-treated mice and sham-operated mice in either the ipsilateral or the contralateral DG ($P > 0.05$; Fig. 6A,B,D). In addition, we examined the progenitor cell proliferation in the SVZ and found that fluoxetine treatment did not influence the number of BrdU-labeled cells in this brain region (in fluoxetine group: 3,520 ± 564 for ipsilateral, 3,318 ± 501 for contralalteral; in vehicle group: 3,326 ± 402 for ipsilateral, 3,178 ± 429 for contralalteral).

To determine the neuronal phenotype of BrdU-positive cells in the DG, brain sections were colabeled with antibodies to BrdU and NeuN (neuronal marker) or BrdU and GFAP (glial marker). We found that ~70% of the BrdU-positive cells in the DG were colabeled with NeuN and ~10% were colabeled with GFAP 4 weeks after the last BrdU injection (Fig. 7), but there was no difference in the percentage of $BrdU^+/NeuN^+$ cells between the fluoxetine- and vehicle-

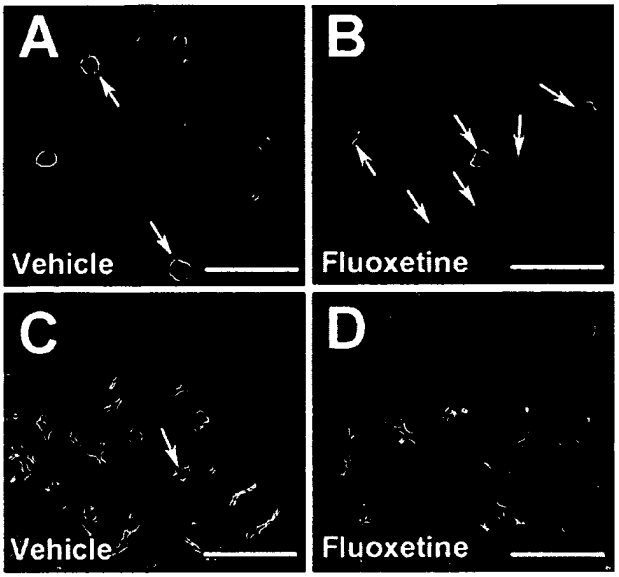

Fig. 7. Neuronal identity of $BrdU^+$ cells in the DG 28 days after the last BrdU injection. The hippocampal sections were doubly stained with BrdU (red) and NeuN (green; **A,B**) or GFAP (green; **C,D**) to show the neuronal phenotype of newborn cells. Arrows indicate double-labeled cells. Scale bars = 50 μm.

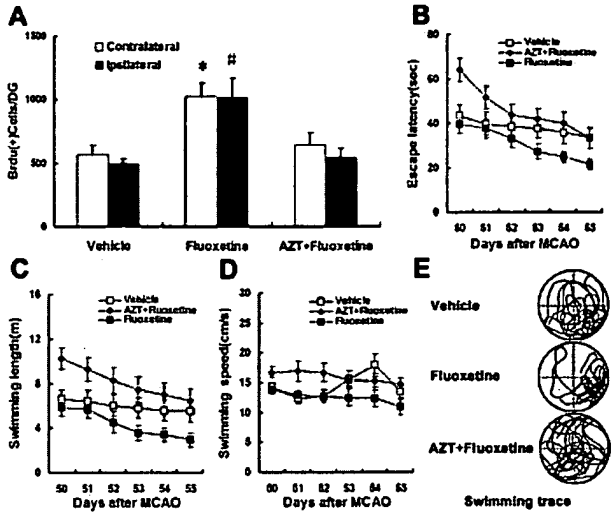

Fig. 8. Disrupting hippocampal neurogenesis abolishes the beneficial effects of fluoxetine on ischemia-induced spatial cognitive deficits. AZT was used to disrupt neurogenesis during the period of fluoxetine treatment. The survival of newborn cells and spatial cognitive deficits were examined 28 days after the last BrdU injection. The number of BrdU-positive cell numbers in the mice treated with fluoxetine combined with AZT (n = 5) were significantly lower than in the mice treated with fluoxetine alone (n = 6) and similar to the numbers in vehicle-treated animals (n = 5), suggesting that the increased neurogenesis by fluoxetine treatment was neutralized by the negative action of AZT (**A**). In the water maze task, AZT completely abolished the effects of fluoxetine on escape latency (**B**), swimming length (**C**), and swimming traces (**E**) compared with the fluoxetine-alone group, and even the speeds of fluoxetine and AZT treatment mice (n = 8) were more rapid than with fluoxetine alone (n = 10) or in vehicle-treated mice (n = 8; **D**). Data are expressed as mean ± SEM. $*P < 0.01$ compared with contralateral DG of vehicle-treated or AZT + FLU (fluoxetine) group; $\#P < 0.01$ compared with ipsilateral DG of vehicle-treated or AZT + FLU group.

treated mice in either ipsilateral or contralalteral regions (ipsilateral: 72.9% ± 5% for fluoxetine, 71.5% ± 6% for vehicle; contralateral: 69.3% ± 6% for fluoxetine; 70.1% ± 4% for vehicle).

Hippocampal Neurogenesis Is Essential for Fluoxetine-Improved Spatial Cognitive Deficits

To confirm the role of hippocampal neurogenesis in the effect of fluoxetine treatment on spatial cognitive performance after MCAO, AZT was used to disrupt neurogenesis during the period of fluoxetine treatment. First, we examined whether fluoxetine-induced neurogenesis is counteracted by AZT 28 days after the last BrdU administration. In the mice treated with fluoxetine combined with AZT, the number of BrdU-positive cells in the DG was significantly lower than that in the mice treated with fluoxetine alone ($P < 0.01$) and was similar to that in vehicle-treated animals (Fig. 8A), suggesting that the increased neurogenesis by fluoxetine treatment was neutralized by the negative action of AZT. Next, we examined whether AZT counteracts the effects of fluoxetine-improved spatial cognitive deficits. In water maze task, AZT completely abolished the effects of fluoxetine on escape latency ($P < 0.05$; Fig. 8B) and swimming length ($P < 0.05$; Fig. 8C) compared with the fluoxetine-alone group; even the speed of the fluoxetine + AZT group was greater than that of the fluoxetine alone group ($P < 0.05$; Fig. 8D). In the spatial probe trials, the swimming traces of fluoxetine-treated mice concentrated in the target zone where the platform had been set. However, the swimming traces of vehicle-treated mice and fluoxetine/AZT-treated mice were uniformly distributed around four zones (Fig. 4E). These results suggest that AZT counteracts the effects of fluoxetine on spatial memory. Thus, hippocampal neurogenesis is required for the beneficial effect of fluoxetine on ischemia-induced spatial cognitive deficits.

DISCUSSION

Fluoxetine is a widely used antidepressant compound, so most of the scientific studies on the drug have concentrated on depression (Santarelli et al., 2003; Cipriani et al., 2005). Here we report that fluoxetine treatment for 4 weeks promoted the survival of newborn cells in the DG and consequently reversed spatial memory impairment after focal cerebral ischemia. However, fluoxetine had no effects on ischemia-induced sensorimotor functional deficits. To the best of our knowledge, this is the first report that fluoxetine can enhance neurogenesis and has beneficial effects on spatial cognitive function following focal cerebral ischemia.

Poststroke spatial cognitive deficit is one of the most common results of ischemic stroke and has received more and more attention. Fluoxetine had actually been applied clinically in ischemic stoke therapy years ago, but mainly to treat poststroke depression (PSD). It was found not only to improve depressive symptoms but also to promote motor recovery of stroke patients (Dam et al., 1996). However, nobody knows whether there are other mechanisms or beneficial effects that fluoxetine may exert on stroke patients apart from improving depressive symptoms. A clinical study reported that a single dose of fluoxetine (20 mg) enhanced motor performance and modulated cerebral sensorimotor activation in stroke patients (Pariente et al., 2001). Several animal experiments have also been performed that aimed to identify how this drug influences recovery of function in ischemic rodents. However, those studies show that fluoxetine treatment failed to improve functional recovery following cerebral ischemia (Jolkkonen et al., 2000; Zhao et al., 2005; Windle and Corbett, 2005). This may be due to the fact that most of those studies used a shorter period of fluoxetine treatment (10–15days), considering that it is enough to alter brain amine concentrations (Jolkkonen et al., 2000), or they focused only on observing sensorimotor functional recovery of ischemic animals (Zhao et al., 2005; Windle and Corbett, 2005). Importantly, recent studies have shown that chronic fluoxetine treatment affects neuroplasticity and can promote hippocampal neurogenesis both in the normal brain and in the depressive brain, and it is chronic, but not acute, fluoxetine treatment that produces the effects. In the present study, we used a long period of fluoxetine treatment (28 days) after cerebral ischemia and examined not only sensorimotor but also spatial cognitive functional recovery as well as hippocampal neurogenesis. Consistently with previous studies (Zhao et al., 2005; Windle and Corbett, 2005), fluoxetine did not have any effect on sensorimotor functional recovery of ischemic animals in the present study. However, the chronic fluoxetine treatment markedly attenuated ischemia-induced spatial learning and memory deficits. The novel findings of our study indicate that fluoxetine may be used clinically to treat poststroke spatial cognitive deficits. Our results were supported by a recent study indicatinhg that fluoxetine treatment improved cognitive impairment after neonatal hypoxic-ischemia (HI) brain injury in rats (Chang et al., 2006). Moreover, a clinical study reported that fluoxetine can enhance memory and cognition of patients with mild cognitive impairment (MCI; Mowla et al., 2007).

The functional benefits of spatial cognitive performance derived from chronic fluoxetine treatment were, at least in part, the result of fluoxetine-induced survival of newborn cells in the hippocampus after ischemic stroke. Hippocampal neurogenesis has been observed in adult mammalian animals (Kuhn et al., 1996; Kornack and Rakic, 1999), and these newborn cells have long been associated with the acquisition of new hippocampus-dependent memory (Shors et al., 2001). Recent data suggest that hippocampal neurogenesis plays a contributory role in functional recovery after cerebral ischemia. Inhibiting hippocampal neurogenesis exacerbates ischemia-induced cognitive impairments (Raber et al., 2004), whereas enhancing hippocampal neurogenesis promotes spatial memory recovery after cerebral ischemia (Liu et al., 2007; Luo et al., 2007). Fluoxetine has been shown to have the ability to regulate neurogenesis in the DG of hippocampus. In in-vitro-cultured neural stem cells (NSCs) derived from hippocampal tissues, fluoxetine increased survival, promoted neurite development, and prevented apoptosis (Chiou et al., 2006). In vivo studies with fluoxetine determined that chronic treatment promoted the proliferation as well as the survival and differentiation of newborn cells in the hippocampus, which was proved to be a requisite for its behavioral effects on depression (Malberg et al., 2000; Santarelli et al., 2003; Sairanen et al., 2005). In the present study, we found that chronic fluoxetine treatment significantly promoted the survival of newborn cells in the DG and that most of these newborn cells matured into neurons, which may have integrated into the hippocampal circuitry and restored impaired spatial cognitive performance. Supporting data from the present study showed that 28 days, but not 14 days, of fluoxetine treatment and another 14 days of drug withdrawal restored impaired spatial cognitive performance in the water maze after ischemic stroke, which is identical to the time for newborn cells to mature into functional neurons (van Praag et al., 2002), supporting a role of hippocampal neurogenesis. Moreover, when the enhanced neurogenesis in the DG in fluoxetine-treated mice was neutralized by AZT treatment, the antidepressant beneficial effects of fluoxetine on spatial cognitive deficits disappeared. Also, the spatial memory improvement could not be the result of reduced ischemic injury, because fluoxetine treatment for 4 weeks starting day 8 after MCAO did not lessen infarct volume. Although fluoxetine dramatically increases 5-HT concentration in the synaptic cleft and 5-HT is involved in learning and memory (Shirahata et al., 2006), the spatial memory improvement could not be due to fluoxetine-induced 5-HT enhancement in the synaptic cleft, because the half-life of fluoxetine is 15–26 hr (DeVane, 1994), and it should disappear from the body and has no effect on 5-HT concentration in the synaptic cleft after 14 days of withdrawal. Taken together, these data suggest that hippocampal neurogenesis plays a critical role in fluoxetine-induced spatial cognitive improvement after ischemic stroke.

Fluoxetine had no effect on ischemia-induced sensorimotor functional deficits in the present observations and in other recent studies (Jolkkonen et al., 2000; Zhao et al., 2005; Windle and Corbett, 2005), which differs from clinical studies suggesting that fluoxetine enhances the motor recovery process after stroke (Dam et al., 1996; Pariente et al., 2001). In clinical studies with fluoxetine, the subjects selected were all patients with poststroke depression (PSD). It is widely believed that PSD has a negative impact on the rehabilitation of stroke (Robinson, 1998). Because the depressive symptoms are alleviated by fluoxetine, patients are more motivated to

engage in voluntary exercise and other social activities, which have been proved to be beneficial for functional recovery after stroke (Endres et al., 2003). However, a depressed mood is unlikely to exist in ischemic animals (Windle and Corbett, 2005). The experimental model of MCAO is not a good model for PSD, unlike the case for human stroke, in which about 60% of patients will develop depression (Dam et al.,1996). A PSD animal model shoud be produced by an MCAO plus chronic mild stress (CMS) regimen (Wang et al., 2008). The difference in depressed mood between patients and animals after stroke may therefore account for the discrepancy between clinical reports and animal experiments.

Several recent studies have indicated that fluoxetine promotes progenitor cell proliferation in the adult DG under physiological (Malberg et al., 2000) or depressive (Malberg and Duman, 2003) conditions. In the current study, however, chronic fluoxetine treatment did not have a significant effect on cellular proliferation and increased progenitor cell survival only in the ischemic hippocampus. We also found this phenomenon in our previous study, which showed that voluntary exercise, which has been reported to enhance progenitor cells proliferation in the hippocampus in normal animal (van Praag et al., 1999), did not enhance progenitor cell proliferation after cerebral ischemia (Luo et al., 2007). Another study of neonatal hypoxic-ischemia (HI) brain injury also reported that chronic fluoxetine treatment could increase specifically progenitor cell survival, but not proliferation in the hippocampus, and improve cognitive functional recovery after HI brain injury (Chang et al., 2006). The discrepancy may be due to the animal conditions used in the experiments. The brain microenvironment caused by acute and destructive cerebral ischemia is dramatically different from that of normal brain or chronic-stress brain. One of the possible reasons is that low BDNF or other neurotrophic factor levels in the DG, caused by the excessive consumption after ischemia, makes progenitor cells in the ischemic brain insensitive to beneficial stimuli. Supporting evidence comes from the findings that BDNF protein is decreased significantly in CA1 at early phases of transient forebrain ischemia (Lee et al., 2004). However, this necessitates further study. As opposed to our findings showing that fluoxetine promoted newborn cell survival, one previous study has indicated that fluoxetine had no effect on ischemia-induced neurogenesis increase in the adult DG (Choi et al., 2007). The discrepancy could be due to differences in objectives, experimental design, and fluoxetine administration. First, the aim of Choi et al. was to test the effect of fluoxetine on ischemia-induced progenitor cells proliferation and survival, so they labeled newborn cells with BrdU at 9 days after cerebral ischemia and administered fluoxetine from day 3 to day 9 after ischemia to test proliferation and from day 3 to day 23 to test survival. However, our purpose was to examine whether fluoxetine enhances progenitor cell proliferation and survival during the recovery period of cerebral ischemia. We labeled newborn cells with BrdU from day 19 to day 21 after ischemia, and administered fluoxetine from day 7 to day 21 to test proliferation and form day 7 to day 35 to test survival. Second, the global cerebral ischemia that Choi et al. used is different from the MCAO-induced focal cerebral ischemia used in our study. Third, the dosage of fluoxetine (5 mg/kg) used by Choi et al. was smaller than the dose we used (10 mg/kg).

The mechanisms underlying the roles of fluoxetine in the survival of newborn cells are not completely understood. The CREB pathway is involved in neuroplasticity and cognition as well as in newborn neuronal survival and differentiation (Lonze and Ginty, 2002; Nakagawa et al., 2002; Mizuno and Giese, 2005). We recently found that CREB activation is a necessary and sufficient condition for the survival of new neurons in the DG after ischemia (Zhu et al., 2004). In recent years, several investigations have shown that the activity of CREB is modulated by chronic antidepressant treatments, particularly by fluoxetine. Chronic fluoxetine treatment up-regulates CREB phosphorylation in the hippocampus of both rodents and humans (Dowlatshahi et al., 1998; Tiraboschi et al., 2004). Moreover, fluoxetine treatment can increase CREB phosphorylation and the survival of newborn neurons in the DG after neonatal HI brain injury (Chang et al., 2006). BDNF, a target gene of CREB, is required for the long-term survival of newborn hippocampal neurons (Sairanen et al., 2005). Fluoxetine has been shown to up-regulate BDNF expression both in normal and in ischemic brain (Russo-Neustadt et al., 2000; Kim et al., 2007). Therefore, the CREB-mediated pathway is very likely to contribute to the fluoxetine-induced survival of newborn cells in the ischemic DG, although other factors, such as neuroplasticity and antiinflammatory action, may also be implicated in the effects of fluoxetine (Abdel-Salam et al., 2004; Chang et al., 2006; Chiou et al., 2006). Our future studies will seek to reveal the mechanisms underlying fluoxetine's effect on the survival of newborn cells after ischemic stroke.

In conclusion, our results demonstrate that chronic fluoxetine treatment promotes neurogenesis in the hippocampus and attenuates ischemic stroke-induced spatial cognitive deficits. Therefore, it may be more advantageous for stroke patients to take fluoxetine not only for prevention or treatment poststroke depression (PSD) but also to benefit to cognitive functional recovery. Our findings raise the possibility that the clinical application of fluoxetine may be expanded to treat stroke patients with spatial cognitive deficits, even if there is no PSD, which will provide a safe and effective treatment of poststroke spatial cognitive deficit.

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